Original Article

Role of calcium and phosphorous concentration as an intrinsic factor in the development of skull fracture following road traffic accidents

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ABSTRACT

Introduction: Traumatic brain injury (TBI) or head injury is one of the leading causes of morbidity and mortality globally. TBI includes a fractured skull as an indicator of insult which can affect the treatment outcome as well. The development of any fracture depends on a combination of factors defining the intrinsic properties of the bone and the extrinsic factors related to the impact. A decrease in bone mass secondary to deficiency of calcium (Ca) and phosphorus (P) can be a significant factor intrinsic to the skull bone, which can modulate the outcome of the impact by increasing the susceptibility of bones towards fractures. We undertook this research to find out whether or not the Ca and P concentration in skull bone has a role to play as an intrinsic factor, in the development of skull fracture following Road Traffic Accidents (RTAs). **Methodology:** In this case–control study conducted for two years, we collected 94 bone samples, i.e. 47 each, from skull bones with head injuries following RTA, with (case) and without (control) fracture of the skull. The elemental analyses for the bony concentration of Ca and P in both the groups were then compared using energy dispersive X-ray **(**EDX). Unpaired *t*-test and Fisher's exact test was used for statistical analysis. **Results:** The elemental analysis of bones provided evidence that suggests that whilst; Ca is the only mineral that appears to have a significant correlation with the development of fracture skull, the overall Ca: P ratio of less than 1.99 increases the chances of skull fracture by 3.9 times. **Conclusions:** Both individual bony Ca concentration and Ca: P ratio can be regarded as important intrinsic factors for the development of skull fracture.

Keywords: Energy-dispersive X-ray spectroscopy, road traffic accidents (RTA), skull fracture

Introduction

Traumatic brain injury (TBI) also known as head injury involves the occurrence of injury to the head and is associated with symptoms or signs of neurological abnormalities, skull fracture, intracranial lesions, and death.[1] TBI is a global health problem and requires attention from researchers and the policy stakeholders. In the USA TBI accounts for more than 50,000 deaths each year and Road Traffic Accidents (RTA) is responsible for 50% of TBI cases.[2] In India, it is one of the leading causes of mortality and morbidity in the young.[3]

Fracture skull is one of the most commonly associated fractures with TBI and can be an indicator of substantial insult to the head with possible injury to the vital contents. While most of these fractures are the result of contact violence, a few of them can

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How to cite this article: Tamuli RP, Saikia B, Sarmah S, Patowary AJ. Role of calcium and phosphorous concentration as an intrinsic factor in the development of skull fracture following road traffic accidents. J Family Med Prim Care 2020;9:2854-9.

also result from non-contact forces. Susceptibility of the skull bone to the external mechanical forces depends on factors like chronological age, sex, scalp thickness, and the variety of bone involved.[4] These properties influencing the morphology and biometry of the skull bone can be commonly categorized as intrinsic factors (IF) of the bone. Other variables like kinetics of the contact and properties of the impacting object can be considered as extrinsic factors, which equally determine the degree of injury in the fractured skull.[4] Fracture skull as an outcome is thus the result of an interface between both these intrinsic and extrinsic factors. Apart from these well-known variables considered as IF, the concentration of calcium (Ca) and phosphorous (P) in the skull bone might also be influencing the degree of injury and thus can be explored as an additional IF. Ca along with P is required for the formation of hydroxyapatite that supports the bone mass and provides physical strength to the bone. Bones that are in a dynamic state serve as a reservoir of Ca. As much as 99% of the body's Ca is present in the bones and teeth as calcium hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$).[5] Phosphorus is essential for the development of bones and teeth and about 80% of the body's phosphorus occurs in combination with Ca.[6] There is much scientific evidence which links the deficiency of Ca and P to a decrease in bone mass, making the bone more vulnerable to post-traumatic fractures. In our study, we investigate the possible role of bony concentration of Ca and P as an IF for the development of fracture skull in cases of death due to RTA.

Factors influencing skull fracture

The types and severity of skull fracture inflicted by given traumatic violence depend to a large extent on the following factors[4]:

1. Intrinsic factors (Physical characteristics of the head):
   - Chronological age
   - Sex
   - Thickness of the scalp and covering hair
   - Thickness and configuration of the skull
   - Elasticity of the bone
2. Extrinsic factors (physical characteristics of the impacting object and kinetics of contact):
   - Shape and size of the contact area
   - Mass of the impacting object
   - Consistency, surface structure, rigidity, the sharpness of the edges
   - Velocity of the head
   - Velocity of the object
   - Angle of incidence

Although all these mentioned factors are well established and they do have a collective approach in modulating the outcome of skull fracture, our prime idea in this study was to explore the mineral content of skull bone as a possible IF for physical strength and will be highlighted *per se*. Being a compact bone skull is more rigid than trabecular bone and can withstand greater pressure with a strain limit of 2% change in initial dimensions. Due to its elastic properties, a trabecular bone can store and

release energy with a strain limit up to 75% deformation before fracture.[7] In traumatic fractures, apart from others, bone mineral density (BMD), a measurement used in the diagnosis of osteoporosis, has also been identified as a risk factor for developing a fracture.[8-11] It was further found that BMD showed a significant association with fracture risk with a 40% decrease for each standard deviation (SD) rise in BMD.[11-13]

Objective of the study

1. To find out the association between the concentration of calcium and phosphorus with a fractured skull.
2. To find out the relationship between calcium and phosphorus ratio with a fractured skull.
3. To find out the probability of skull fractures in adults above 30 years of age.

Methodology

The study was approved by the Institute Ethical Committee and samples were collected only after taking informed consent from relatives of the deceased.

Study Period: Two Years

Study design: Case-control study

Sample size: A total number of 94 (ninety-four) bone sample, i.e. 47 samples (case) from death due to head injury following RTA with a fractured skull and 47 samples (control) from death due to head injury following RTA without a fractured skull.

Sample Collection: Bone samples were collected from the site of skull fracture (cases) and the site of head injury without fracture of skull (control). To maintain standardization the samples (both cases and control) were taken only from adult (19–44 years) male and were collected from depressed fracture sites from an area adjacent to the squamous part of the temporal bone, as it is one of the most common sites involved in a fractured skull. All samples were kept in accordance subjected to elemental analysis using EDX in a cool and dry environment.

Inclusion criteria:
1. All confirmed cases of adult male death due to head injury with fracture of skull (Cases) following RTA.
2. All confirmed cases of adult male death due to head injury without fracture skull (Control) following RTA.

Exclusion criteria:
1. Cases with a history of drug abuse/use, for possible altered bone metabolism.
2. Death in cases of skull fracture following RTA, for reasons other than a head injury.
3. Female deaths following RTI
4. Cases of deaths other than adults.
5. Cases without relevant history.
6. Cases without proper consent.

Instrument Used: SEM-EDX analyzer, model 758; DET area: 10 m/m²; Window: ATW2; Resol at 5.9 keV: 137 eV; BIAS: -500 V; OXFORD Instruments at Tezpur University, Assam, India.

Statistics: GraphPadInStat, Versiosdevn 3.05 and Microsoft excel

Energy dispersive X-ray (EDX) spectroscopy

EDX spectroscopy is used for multielemental quantification and is useful as a semiquantitative tool for the analysis of Ca and P in bone and dentine.[14-16] In a study done by Tzaphlidou M and Zaichick V on rib bone of healthy humans the mean values (mean ± SD) for the concentration of Ca and P was found to be 19.3 ± 4.5% and 8.42 ± 2.14% of dry bone weight respectively with a Ca: P ratio of 2.33 ± 0.34. A minimum acceptable value that was found to be 1.99 (2.33-0.34 = 1.99) for Ca: P ratio was thus adapted from Tzaphlidou M and Zaichick V in our study.[17]

Statistical tests performed: Unpaired *t*-test (to calculate "p" value)

Fisher's exact test (to calculate the Odds ratio (OR)

Results

Estimation of calcium and phosphorus in the fractured skull (case)

Estimation of Ca and P in skull bones with fracture showed variable results; the value of calcium varied from 1.06 to 28.54 (Mean 12.0619 and SD ± 6.090), whereas the value of *P* fluctuated between, 0.73 to 12.9 (mean 6.9111, SD ± 3.010) [Tables 1 and 2]. However, individual variations with a high level of deposited calcium (28.54) in skull bone with fracture were also highlighted [Figure 1].

Estimation of calcium and phosphorus in non-fractured skull bones (control)

Variable results were also observed in the estimation of Ca and P in skull bones without fracture. Levels of deposited calcium and phosphorus in non-fractured groups were as high as 28.78 and 12.89 and as low as 1.87 and 0.37, respectively [Figure 2].
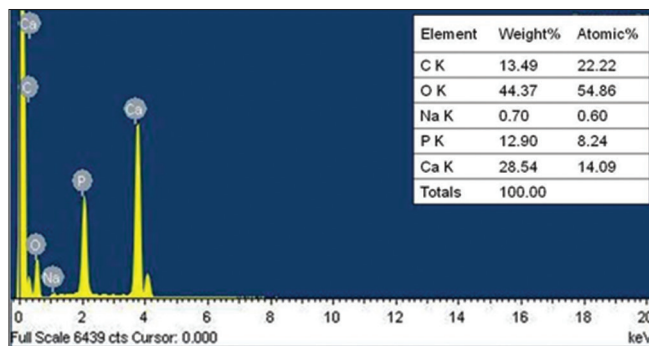
A comparison of values of deposited Calcium (Ca) in both fractured and non-fractured skull bones is provided in Table 1. It is evident [Table 1] that there was a very significant difference between values of deposited Ca in both cases (fractured) and controls (non-fractured) groups ($P = 0.0071$).

Comparison of values of deposited Phosphorus (P) in both fractured and non-fractured skull bones can be seen in Table 2. It is evident [Table 2] that there were no significant differences between the values of deposited P in both cases (fractured) and controls (none fractured) groups ($P = 0.1409$).

To find out the relationship between Ca: P ratio and fracture of the skull, values were tabulated [Table 3] and OR was calculated.

After calculation of OR, the result can be emphasized as the fracture of skull was 3.9 (3.872) times more common if Ca: P ratio was below 1.99 (OR = 3.872, 95% confidence interval (CI) =1.364 to 10.992, and $P = 0.0153$), which was statistically significant.

The association between fracture skull and chronological age above 30 years was calculated [Table 4].

Table 1: Levels of deposited Ca in both case and control groups

	Fracture Ca	Non fracture Ca
Mean	12.0619	15.6234
Standard deviation	±6.090	±6.448
P	0.0071 (Very Significant)	
T	2.753 (df=92)	

Table 2: Levels of deposited P in both case and control groups

	Fracture P	Non fracture P
Mean	6.9111	7.8511
Standard deviation	±3.010	±3;125
P	0.1409 (Not significant)	
T	1.485 (df=92)	

Element	Weight%	Atomic%
C K	13.49	22.22
O K	44.37	54.86
Na K	0.70	0.60
P K	12.90	8.24
Ca K	28.54	14.09
Totals	100.00	

Figure 1: Figure of elemental analysis sowing a high level of deposited Ca in a 49 year old man showing fracture of skull (Inset shows complete elemental analysis of the same individual)

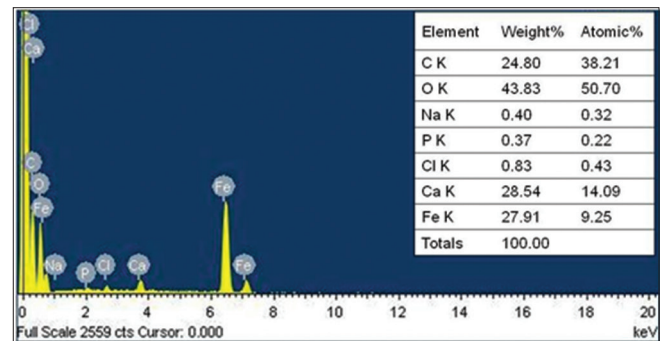| Element	Weight%	Atomic%
C K	24.80	38.21
O K	43.83	50.70
Na K	0.40	0.32
P K	0.37	0.22
Cl K	0.83	0.43
Ca K	28.54	14.09
Fe K	27.91	9.25
Totals	100.00	

Figure 2: Figure of elemental analysis showing a low level of deposited Ca and P in a 38 year old male without skull fracture (Inset shows complete elemental analysis of the same individual)

Table 3: Showing number of cases with normal and abnormal Ca: P ratio in both case and control groups

	Fracture	Non fracture
Abnormal Ca: P (<1.99)	41 (a)	30 (b)
Normal Ca: P (>1.99)	6 (c)	17 (d)

Table 4: Showing number of cases above and below 30 years showing fracture of skull

	Fracture	Non fracture
Adult (>30)	22 (a)	26 (b)
Young (<30)	25 (c)	21 (d)

From the OR, it was highlighted that skull fracture was not having any association with the chronological age of above 30 years. However, this result was without any statistical significance. (Odds ratio = 0.7108, CI = 0.3156 to 1.601, *P* = 0.5362).

Discussion

TBI is a major public health problem worldwide, which needs much attention to research.[3] The presence of fracture in TBI is an indicator of underlying injury and may affect the treatment prognosis. However, the type and site of depressed skull fractures were reported as not to be statistically influencing the treatment outcomes.[18] In our study, the samples were taken from the site of a depressed fracture adjacent to the squamous part of the temporal bone. Bone is composed primarily of Ca and P in the form of hydroxyapatite crystals deposited in a collagen matrix.[19] It is a metabolically active tissue with constant turnover regulated by cellular activities that reabsorb and form bone in such a balanced way that the total bone mass remains the same.[20] To either increase or decrease net bone mass, these cellular processes must become functionally uncoupled. By contrast, the major mineral ions of bone (calcium, phosphorus and, magnesium) play a more passive role in bone mass changes.[21]

Many variables can affect the fracture properties of bone. Some, such as porosity and mineral content, are intrinsic to the material; others, such as strain rate or complexity of loading, are extrinsic.[22] In our study, we found that although there was a significant difference in mineral content between the fractured and non-fractured groups, it only concerned the individual bony concentration of Ca, while the individual bony P concentration between the two groups was not having any significant difference. While investigating factors related to depressed skull fractures and their treatment outcome, it had been found that the type, site of the fracture, age, and sex distribution was not significantly influencing the outcome.[18] Even though conducted in post mortem cases of RTA; our study emphasizes the bony concentration of Ca as an important factor influencing the degree of injury, which might be collateral in indicating the treatment outcome in cases of TBI. Moreover, we found statistically significant evidence that the fracture of the skull following RTA was 3.9 times more common if the Ca: P ratio

was below 1.99. Such important rationales can now contemplate our understanding of the overall mechanism of the fractured skull. While the "Ca" identifies itself as the only mineral affecting the fracture properties to be precise, the overall "Ca: P ratio" of the mineral composition itself can now be recognized as an additional influencing factor intrinsic to the material (skull bone).

The theoretical fraction of Ca in hydroxyapatite is 40.3% and P is 18.4%.[23] However, the Ca and P content of hydroxyapatite in the human bone may not correspond to these values, as shown in studies employing chemical or instrumental neutron activation analysis (INAA), where the values vary between 18.5–62% for Ca and 8.7–27% for P.[23,24] EDX allows for parallel quantification of major elements present in individual trabeculae and cortices. A study done by Akesson K *et al.* comparing EDX, INAA, and inductively coupled plasma emission spectroscopy (ICPES) found that the Ca concentration to be slightly higher using EDX when compared to other techniques like INAA and ICPES.[25] In our study we found the mean concentration of both Ca and P to be significantly lower in both the groups when compared to Akesson K *et al.* Whether or not this low concentration is due to factors like geographical distribution, diet or Body Mass Index is a matter of further investigation. Yet analytical method using EDX for quantifying the major mineral components of bone is a method of good precision and accuracy and it correlates well with other quantitative methods.[25] As mentioned earlier a minimum acceptable value for Ca: P ratio in our study was adapted as per Tzaphlidou and Vladimir Zaichick owing to the availability of a minimum value of Ca: P ratio, i.e. 1.99 which was acceptable for a valid result.[17]

Another study with an electron probe microanalysis for the concentration of Ca and P in Tibial bone comparing post-traumatic osteopenia and control cases found the concentration of Ca as well as of P was lower in osteopenia as compared to normal control subjects. The Ca: P ratio was also seen to be low in post-traumatic osteopenia.[26] No fracture marks indicative of previous trauma was observed in any of our cases.

While it was seen that age-related bone loss is a general phenomenon and is further found to be greater in the case of females than in males,[27] yet treatment outcome of depressed fracture was not significantly affected by age and sex of the individual.[18] Although the relationship of sex with a fractured skull following RTA couldn't be analyzed in our study to comment on, there was no correlation between the skull fracture following RTA and chronological age above 30 years of age. To eliminate the IF variables like the weight of the head, area of impact, the thickness of the vault, and visco-elastic properties of the human scalp, the bone samples were taken from the squamous part of the temporal bone in an adult male. However, the effect of extrinsic factors like impact velocity, geometry, and compliance of the impact material together with the above mentioned intrinsic factors together brings about an overall cumulative outcome in a skull fracture.[28,29]

Limitations

- The study carried out in one of the premier health institutes of Assam, India might not exactly be representing the global Ca and P concentration in skull bones.
- To extend the validity of individual bony Ca concentration and Ca: P ratio as an intrinsic factor for fractured skull, a wider population group and a wider range of target compliance study is advisable.
- External factors like physical characteristics of the impacting object and kinetics of contact become crucial and should also be investigated for an overall fracture outcome.
- Assessment of concentration of other minerals like Magnesium and Aluminum can be done for further validation.
- Additional studies comparing the results of EDX quantification with Neutron Activation Analysis (NAA) or Inductively Coupled Plasma Emission Spectroscopy (ICPES) determining concentration of Ca and P should be carried out for further validation.

Conclusions

- There was a decreased individual bony Ca concentration observed in the fractured group as compared to non-fractured ones, the difference between which was statistically significant. Thus, individual bony Ca concentration qualifies as one of the IF for the development of fracture, lower level of which increases the susceptibility of the skull towards post-traumatic fracture.
- There was no significant difference in individual P concentration between the two groups. Thus, the bony P levels don't seem to modulate the mechanism of fractured skull.
- It was found that a value of Ca: P ratio of <1.99, increases the chances of fracture skull by 3.9 times. Thus it can be argued now that apart from the individual bony Ca concentration, the overall Ca: P ratio also identifies itself as an important intrinsic factor influencing the development of fractured skull.

Conflicts of interest

There are no conflicts of interest.

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